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(72) The inventors of this invention in the sense of being the actual devisers thereof within the meaning of Section 16 of the Patents Act 1949 are TAGE KJAER NIELSEN of 101 Rørløkken, DK-2730 Herlev, and ERIK KJAER MARKUSSEN of 18, Tornekrogen, DK-3500 Vaerløse, Denmark, both Danish subjects.

(54) IMPROVEMENTS IN OR RELATING TO THE PRODUCTION OF ENZYME PREPARATIONS

(71) We, NOVO TERAPEUTISK LABORATORIUM A/S, a Danish company of 215, Nordre Fasanvej, 2200 Copenhagen N, Denmark, formerly of 115, Fuglebakkevej, 2200 Copenhagen N, Denmark, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to a process for the production of enzyme preparations consisting of uniformly sized solid spheres.

In this specification and in the claims the expression "pellets" is intended to cover not only normal pellets, but also extruded shaped bodies having an elongated structure, for example, a spaghetti-like structure.

It is known to convert an extruded material into uniformly sized solid spheres by supplying the extruded pellets to a container with stationary solid side walls and a rotatably mounted bottom friction plate rotating with a speed up to 1800 rpm. This spherroidising is caused by centrifugal force and friction and has been performed in machines sold under the name MARUMERIZER. The word "MARUMERIZER" is a Trade Mark.

We have now found that this spheroidising process is very useful in connection with enzyme preparations, particularly for use in the detergent industry, for example, preparations comprising enzymes and additives normally used in washing and cleaning compositions, when the process is carried out with certain extruded enzyme-containing pellets. These pellets are produced in a conventional manner from a mixture of an enzyme-containing powder and one or more of the compounds: polyglycols, fatty alcohols, ethoxylated fatty alcohols, higher fatty acids, mono-, do- and tri-glycerol esters of higher fatty acids, and alkylarylethoxylates.

Accordingly, the process of the invention comprises subjecting enzyme-containing pellets prepared by extrusion from a mixture of from 3 to 95 per cent of solid enzyme-containing powder comprising, if desired, an enzyme stabiliser, and from 97 to 5 per cent of one or more of the aforesaid compounds to a spheroidising process using a rotational speed of up to 2000 rpm in an apparatus causing centrifugal and frictional forces to be applied to the pellets.

The enzyme preparations which can be produced by the process of this invention consist of particles of practically uniform size suitable for the intended industrial uses. The particles are substantially dust-free and show a sufficient mechanical strength for handling without the formation of dust. The particles also show sufficient flow properties for transportation in factories.

In the following Examples, rotational speeds of up to 800 to 900 rpm are used during the spheroidisation, but speeds up to 2000 rpm may be employed.

In accordance with a preferred embodiment of the invention the spheroidizing process is carried out in a machine of the type having a cylindrical container with a stationary solid side wall and a rotatably mounted bottom friction plate rotating with a speed of up to 1800 rpm, such as a "MARUMERIZER".

The enzyme powder in addition to the enzyme itself preferably contains suitable additives such as enzyme stabilisers, fillers; gelatin and casein acting as substrates for the enzymes may be mentioned as enzyme stabilizers, and examples of fillers are inorganic salts, such as sodium chloride, sodium tripolyphosphate, tetrapotassium pyrophosphate and sodium sulphate, cellulose powder, starch powder, cellulose derivatives, starch derivatives or starch decomposition products and water-soluble silicates.

The extrusion is preferably carried out at

a temperature in the neighbourhood of the softening point of the compounds added to the enzyme powder. If the melting point of the compounds is between 30 and 40°C the extruded product usually has a tendency to become somewhat sticky. Melting points above 60°C will require preheating of the mixture to be extruded.

The ratio between the enzyme powder and the added compounds depends on the enzymatic activity of the enzyme powder and on the desired enzymatic activity of the final spheroidised product.

In order to prevent a tendency of the spheroidised particles to adhere to each other use may be made of powdering, for instance with inorganic salts or oxides such as titanium dioxide.

The following Examples illustrate the process of the invention. In these Examples, there was used an enzyme concentrate called ALCALASE which is a commercial product and contains a proteolytic enzyme together with some inactive organic matter, and inorganic salts, mainly sodium sulfate, and an example is also present in which a fungal α-amylase is used. The word "ALCALASE" is a Trade Mark.

Furthermore, in the present process use may also be made of other enzymes, for example proteolytic enzymes produced as described in our co-pending Cognate British Patent Applications Nos. 45046/67 and 35921/68 (Serial No. 1,243,784) by aerobic cultivation of protease-forming species of the genus Bacillus on a nutrient medium having a pH within the range of 9 to 11 and maintaining during the main period of cultivation a pH in the said medium between 7.5 and 10.5, the said proteolytic enzymes showing a proteolytic activity of 80 to 100 per cent of maximum activity when measured at pH 12 by the Anson hemoglobin method carried out in the presence of urea.

Examples of enzymes which may be successfully employed in the process of the invention, are proteinases and amylases as mentioned above, and also lipases, milk-coagulating enzymes, cellulases and hemicellulases, glucoseisomerase, amyloglucosidase and pectinases.

The spheroidising treatment referred to in the foregoing may be carried out using a material prepared in an axial or a peripheral extruder. However, in the case of an end product having a low bulk density, it is preferred to use a peripheral extruder in which the material to be extruded is subjected to a relatively low extrusion pressure and thus becomes relatively little compressed.

Especially for detergent preparations, it is possible to prepare a spray-cooled or prilled product by spraying a melt of polyethyleneglycol or a non-ionic surfactant with a suspended enzyme in a tower through which a cold stream of air is introduced, bringing the sprayed melt to solidify to small spheres or the like. It is a drawback of this method that costly apparatus is used, and that a relatively large amount of waxy substance (about 50%) is used. In the process of the present invention the amount of waxy substance necessary for preparing a stable granulate is considerably smaller. Furthermore, a spray-cooled product can only be coloured by colouring in grain, whereas the product of the invention may be surface-coloured during or after the spheronization.

The percentages in the Examples are per cent by weight.

Example 1

There is produced a premix consisting of

12% ALCALASE
67% Sodium chloride
21% NONIPOL CS-50 (Melting Point 47—51°C)

NONIPOL CS-50 is fatty alcohols with 16 to 18 C-atoms and ethoxylated with 50 moles of ethylene oxide.

This premix was extruded in the conventional manner through a 0.7 mm screen. By the friction in the extrusion equipment the mixture is heated to a temperature round 40°C so that it becomes plastic. Cold air was blown through the extrudate immediately after the extruder to prevent adhesion of the pellets which were then placed on a vibrating screen to separate pellets having a diameter above 3 mm. The remaining pellets were then spheroidised in a MARUMERIZER, at the beginning at a speed of 400 rpm while powdering with titanium dioxide, and finally at a speed of 800 rpm. Any traces of dust from the powdering substance can be removed by screening.

The final product has the following properties:

Proteolytic activity	0.48 Anson units/g
Particle size	0.7 mm
Bulk Weight	about 1.0 g/cm³

The product is dust-free and soluble in aqueous media. The Anson method of measuring proteolytic activity is described in J. Gen. Physiol., 22, 79—89 (1938).

Example 2

There is produced a premix of the following composition:

30% ALCALASE
10% Polyethyleneglycol 6000 (Melting Point 60 to 63°C)
60% Sodium tripolyphosphate (Marchon type D)

The word "MARCHON" is a Trade Mark.

This mixture was extruded as described in Example 1, except that it was moistened with 2% of water to make the extrusion possible. The extrudate was then spheroidised as described in Example 1.

The final product showed the following properties:

Proteolytic activity	1.3 Anson units/g
Particle size	0.7 mm
Bulk Weight	about 1.0 g/cm³

The product is soluble in aqueous media.

Example 3

There was produced a premix consisting of

80% Fungal α-amylase
20% Polyethyleneglycol 2000 (Melting Point 48—52°C)

The mixture was lubricated with 13% of polyethyleneglycol 600.

The lubricated mixture was extruded through a 0.9 mm screen and spheronized at a speed of 900 rpm.

3% of anhydrous sodium sulphate was added during the spheroidisation to prevent lumping in the MARUMERIZER.

Into the final product was mixed 0.25% of AEROSIL to make it non-sticky. AEROSIL is a highly dispersed silicon dioxide powder with a particle size about 10 mμ. The word "AEROSIL" is a Trade Mark.

Product properties:

Enzymatic activity	2000 FAU/g
Particle size	0.8—0.9 mm
Bulk density	0.9 g/cm³

The α-amylase activity (FAU/g) is measured according to Cereal Chem., *16*, 712 (1939), the method described being modified so that the following equation may be used for calculations:

1000 SKB units (pH 4.7)≅37 FAU

for fungal α-amylase.

Example 4

A mixture of the composition

10.0% ALCALASE
17.0% Polyethyleneglycol 2000 (Melting Point 48—52°C)
3.5% Polyethyleneglycol 600 (Melting Point 20—25°C)
69.5% Sodium chloride

was moistened with 1% of water, extruded through a 0.8 mm screen and spheroidised as described in Example 1.

The final product showed the following properties:

Proteolytic activity	0.5 AU/g
Particle size	app. 0.8 mm
Bulk density	app. 1 g/cm³

Example 5

A premix of the composition

ALCALASE	58%
Polyethyleneglycol 2000 (Melting Point 48—52°C)	17%
Polyethyleneglycol 600 (Melting Point 20—25°C)	3%
Sodium chloride	22%

was extruded as described in Example 1, except that it was moistened with 2% of water to make the extrusion possible.

The extrudate was spheroidised at 900 rpm.

The final product showed the following properties:

Proteolytic activity	6.0 AU/g
Particle size	0.7 mm
Bulk density	about 1.0 g/cm³

WHAT WE CLAIM IS:—

1. A process for the production of an enzyme preparation consisting of substantially uniformly sized solid spheres, which process comprises subjecting enzyme-containing pellets prepared by extrusion from a mixture of from 3 to 95 weight per cent of solid enzyme-containing powder comprising, if desired, an enzyme stabiliser, and from 97 to 5 weight per cent of one or more of the following compounds: polyglycols, fatty alcohols, ethoxylated fatty alcohols, mono-, di- and triglycerol esters of higher fatty acids and alkylaryl-ethoxylates, to a spheroidising process using a rotational speed of up to 2000 rpm in an apparatus causing centrifugal and frictional forces to be applied to the said pellets.

2. A process according to Claim 1, wherein the melting point of the compound(s) is (are) in the range of from 40°C to 60°C.

3. A process according to Claim 1 or 2, wherein the spheroidising process is carried out using a powdering agent preventing adhesion between the spheroidised particles.

4. A process according to Claim 3, wherein the powdering agent is an inorganic salt or an inorganic oxide.

5. A process according to any one of the preceding claims, wherein the spheroidising process is carried out in a machine of the type having a cylindrical container with a stationary solid side wall and a rotatably mounted bottom friction plate rotating with a speed up to 1800 rpm.

6. A process according to any one of the preceding claims, wherein the solid enzyme

powder used comprises a stabilizer selected from gelatine and casein.

7. A process according to any one of the preceding claims, wherein there is employed an enzyme powder in which the enzyme is selected from proteases, amylases, amyloglucosidase and isomerases.

8. A process according to any one of Claims 1 to 6, wherein there is employed an enzyme powder in which the enzyme is selected from bacterial protease in powder form and prepared from *Bacillus licheniformis*, fungal α-amylase and proteolytic enzymes prepared by aerobic cultivation of protease-forming species of the genus Bacillus on a nutrient medium having a pH within the range of 9 to 11 and maintaining during the main period of cultivation a pH in the said medium between 7.5 and 10.5, the said proteolytic enzymes showing a proteolytic activity of 80 to 100 per cent of maximum activity when measured at pH 12 by the Anson hemoglobin method carried out in the presence of urea.

9. A process for the production of enzyme preparations consisting of substantially uniformly sized solid spheres, substantially as described in foregoing Example 1.

10. A process for the production of enzyme preparations consisting of substantially uniformly sized solid spheres, substantially as described in foregoing Example 2.

11. A process for the production of enzyme preparations consisting of substantially uniformly sized solid spheres, substantially as described in foregoing Example 3.

12. A process for the production of enzyme preparations consisting of substantially uniformly sized solid spheres, substantially as described in foregoing Example 4.

13. A process for the production of enzyme preparations consisting of substantially uniformly sized solid spheres, substantially as described in foregoing Example 5.

14. An enzyme preparation consisting of substantially uniformly sized solid spheres, whenever prepared by the process of any one of the preceding claims.

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